

Amendments to the Specification:

Please amend the specification as shown:

Please delete paragraph [0163] on page 37, and replace it with the following rewritten paragraph:

[0163] Figure 2: Alignment of the CAH gene from *Myrothecium verrucaria* (**SEQ ID NO: 21**) with a new cyanamide tolerance gene isolated from *Aspergillus* (CAH-H1) (**SEQ ID NO: 22**) and a non-functional yeast CAH homolog (CAH-H2) (**SEQ ID NO: 23**).

Please delete paragraph [0164] on page 37, and replace it with the following rewritten paragraph:

[0164] Figure 3: Alignment between a new ubiquitin-like promoter (UbiN) (**SEQ ID NO: 25**) and the corresponding partial sequence of the sugarcane Ubiquitin-4 promoter (**SEQ ID NO: 24**).

Please delete Table 1 on page 42, and replace it with the following rewritten table:

Table 1. "Border" and "Border-Like" sequences

<i>Agrobacterium</i> T-DNA borders	
TGACAGGATATATTGGCGGGTAAAC (SEQ ID NO. 4226)	<i>Agro. nopaline</i> strains (RB)
TGGCAGGATATATTGTGGTGTAAC (SEQ ID NO. 4327)	<i>Agro. nopaline</i> strains (LB)
TGGCAGGATATATACCGTTGTAATT (SEQ ID NO. 4428)	<i>Agro. octopine</i> strains (RB)
CGGCAGGATATATTCAATTGTAATT (SEQ ID NO. 4529)	<i>Agro. octopine</i> strains (LB)
TGGTAGGATATATACCGTTGTAATT (SEQ ID NO.16)	LB mutant
TGGCAGGATATATGGTACTGTAATT (SEQ ID NO.17)	LB mutant
YGRYAGGATATATWSNVBKGTAAWY (SEQ ID NO.18)	Border motif
Border-like sequences	
TGACAGGATATATGGTAATGTAAAC (SEQ ID NO.19)	potato (border-like sequence)*
TGGCAGGATATATACCGATGTAAAC (SEQ ID NO.20)	potato (border-like sequence)*

Y= C or T; R= A or G; K= G or T; M= A or C; W= A or T; S= C or G; V= A, C, or G; B= C, G, or T.

*potato border-like sequences were obtained and isolated according to the presently-described inventive methods.

Please delete paragraph [0284], beginning on page 79 and bridging to page 80, and replace it with the following rewritten paragraph:

[0284] A putative fungal cyanamide tolerance gene was amplified from *Aspergillus* DNA with HotMaster Taq DNA Polymerase (Eppendorf). The primer pair used in these reactions was 5'- TCTAGATGTCACAGTACGGATTTGTAAG -3' (SEQ ID NO: 30), and 5'- GGTCACCTCACTGCCCCATCAGGGTGCCGGCTTC -3' (SEQ ID NO: 31). The amplified fragments were both inserted into the yeast expression vector pNMT1-TOPO (Invitrogen) and the bacterial vector pGEM-T (Invitrogen). Sequence analysis of the new cyanamide tolerance gene inserted into pGEM-T (designated *CAH-H1*; see SEQ ID No.: 1) revealed less than 50% homology with both the previously identified *Myrothecium verrucaria* cyanamide hydratase (*CAH*) gene (Maier-Greiner et al., *Angew Chem Int Ed Engl*, 30: 1314-1315, 1991), and a *CAH* homolog of the highly cyanamide-sensitive species *Saccharomyces cerevisiae* (Figure 2). The PNMT1-TOPO vector carrying *CAH-H1* was introduced into *Saccharomyces pombe* by using the S.c. EasyComp Transformation Kit (Invitrogen). Functional activity of the homolog was demonstrated by growing transformed cells on Edinburgh minimal medium (Invitrogen) containing 100 mg/L ampicillin and 50 mg/L cyanamide at 30°C. After 4 days, numerous colonies were observed on plates containing *S. pombe* cells transformed with pNMT1:*CAH-H1*, whereas no colonies were observed on pNMT1 control plates. The new cyanamide tolerance gene can be used as selectable marker gene for plant transformation by inserting it between a functional promoter and terminator, and introducing the resulting expression cassette into plant cells.

Please delete paragraph [0285] on page 80, and replace it with the following rewritten paragraph:

[0285] A second new *Cah* homolog, *i.e.*, a “cyanamide resistance gene” that comprises the nucleotide sequence depicted in SEQ ID NO.:12, was isolated from *Aspergillus terricola* using *Cah-H1*-derived primers, 5'-ATG TGT CAG AAC GAA GTT GAA GT -3' (**SEQ ID NO: 32**) and 5'-GGT CAC CTC ACT GCC CAT CAG GGT GCC GGC TTC-3' (**SEQ ID NO: 31**).

Please delete paragraph [0286] on page 80, and replace it with the following rewritten paragraph:

[0286] Sequence analysis of the amplified gene revealed the presence of a small intron located within the coding sequence. This intron was removed by ligating two gene fragments, amplified with the primer pairs 5'-TCT AGA TGT GTC AGA ACG AAG TTG AAG-3' (**SEQ ID NO: 33**) and 5'-GTA TAC TCG CAT GGA GTG ATT G-3' (**SEQ ID NO: 34**), and 5'-GTA TAC CAC TAC GGA ATG GCT ATC ACA AAG CAG CAG-3' (**SEQ ID NO: 35**) and 5'-CTG CAG TCA CTG CCC ATC AGG GGT G-3' (**SEQ ID NO: 36**). The predicted protein encoded by this new cyanamide resistance gene, *i.e.*, the protein sequence depicted in SEQ ID NO.:13, shares 58% identify with the known *Cah* gene.

Please delete paragraph [0288] on page 81, and replace it with the following rewritten paragraph:

[0288] About a third of the plants (84 of 250) displayed a high level of tolerance, whereas the remainder of the plants developed severe leaf necrosis. The cyanamide-tolerant plants were grown to maturity, and DNA was then extracted from flowers of these plants for PCR analysis. Using the *CAH*-specific primer pair 5'- CCA ACG GAT GGA CTG CCG TTC CAG TC -3' (SEQ ID NO: 37), and 5'- CAT GGA GTG ATT GTA GGT TTC GGG AC -3' (SEQ ID NO: 38), a 180-bp DNA fragment was amplified successfully from DNA of all of cyanamide-tolerant plants, indicating that the analyzed flowers contained the *CAH* gene stably integrated into the genomes of at least some of their cells. Thus, the data demonstrate that the *CAH* gene is an effective new screenable marker gene.

Please delete paragraph [0294] on page 83, and replace it with the following rewritten paragraph:

[0294] 5' TCTAGAATGTGCCAAAACGAGGTGGAGGTGAACGGCTGGACCTCCATGC
CAGCCAACGCCGCGCCATCTTCGGCGACAAGCCATTCATCAAC -3' (SEQ ID NO: 39)

Please delete paragraph [0295] on page 83, and replace it with the following rewritten paragraph:

[0295] 5' GTAGTCGAGGGTCTTGGCCACCACTGGGTCGTCGAATGGGAACCTGATCTC
CTCGATGGAGAGGGCCTTTGGCTCGTTGATGAATGGCTTGTGCGCCGAAG-3' (SEQ ID NO: 40)

Please delete paragraph [0296] on page 83, and replace it with the following rewritten paragraph:

[0296] 5' GTGGTGGCCAAGACCCTCGACTACGCCAAGGCCGTGCTCCACCCAGAG
ACCTTCAACCACTCCATGCGCGTGTACCACTACGGCATGGCCATCACCAAG-3' (SEQ ID NO: 41)

Please delete paragraph [0297] on page 83, and replace it with the following rewritten paragraph:

[0297] 5' GAGGTCGTGGAGGAGGCAGGTGAGGGCCAGGTGATTGGGGAGAG
GGCGGCGGCTTGCTCTGGGAATTGTTGCTTGGTGATGGCCATGCCGTAGTG-3' (SEQ ID NO: 42)

Please delete paragraph [0298] on page 83, and replace it with the following rewritten paragraph:

[0298] 5' CTCACCTGCCTCCTCCACGACCTCGGCACCGCCGAGGAGAACCTCACC
GCCACCCGCATGTCTTCGACATCTACGGCGGCATCAAGGCCCTCTCCGTG-3' (SEQ ID NO: 43)

Please delete paragraph [0299] on page 83, and replace it with the following rewritten paragraph:

[0299] 5' GCCTCGGCGGCGGCCTCGGCTTGGTCCACGGTGGCGCCGAAGTCCTTGAG
CACGGAGAGGGCCTTGATGCCGCCGTAG-3' (SEQ ID NO: 44).

Please delete paragraph [0300] on page 84, and replace it with the following rewritten paragraph:

[0300] The product of this PCR was used for a second PCR with the primers 5'-
TCT AGA ATG TGC CAA AAC GAG GTG-3' (SEQ ID NO: 45) and 5'- GCC TCG GCG
GCG GCC TCG GCT TGG TC -3' (SEQ ID NO: 46).

Please delete paragraph [0302] on page 84, and replace it with the following rewritten paragraph:

[0302] 5' GCCGCCGAGGCCATCATCCGCCACGAGGACATGGGCGTGGACGG
CACCATCACCTACATCGGCCAACTCATCCAACCTGCCACCACCTACGACAACAC-3' (SEQ ID NO: 47)

Please delete paragraph [0303] on page 84, and replace it with the following rewritten paragraph:

[0303] 5' GTGTTGATTTGGGCGGGTCTCGTCGTGCACGAGCTTGCCGAAGTCCTT
CACGTGTGGGTGGAAGCCGGTGTGTCTAGGTGGTGGCGAGTTG-3' (SEQ ID NO: 48)

Please delete paragraph [0304] on page 84, and replace it with the following rewritten paragraph:

[0304] 5' GACGAGACCCGCGCCCAAATCAACACCGCCTACCCACGCCTCAAGTGGTG
CACCTTCTTCTCCGGCGTGATCCGCAAGGAGGAGACCATCAAGCCATGGT-3' (SEQ ID NO: 49)

Please delete paragraph [0305] on page 84, and replace it with the following rewritten paragraph:

[0305] 5' CTGCAGTCATTGGCCGTCTGGGGTGCCGGCCTCGATCTCCTTGTCGAAGTC
CACGAGGTGGGTGGAGTGGCACCATGGCTTGATGGTCTCCTCCTTG-3' (SEQ ID NO: 50)

Please delete paragraph [0306] on page 84, and replace it with the following rewritten paragraph:

[0306] The product of this PCR was used for a second PCR with the primers 5'-GCC GCC GAG GCC ATC ATC CGC CAC G -3' (SEQ ID NO: 51) and 5'- CTG CAG TCA TTG GCC GTC TGG AGT G -3' (SEQ ID NO: 52). A binary vector containing the new synthetic cyanamide resistance gene of SEQ ID NO. 14 was driven by a strong promoter and can be used to generate transgenic plants that display greater levels of cyanamide tolerance than is possible with a similar construct containing either *Cah* or the new cyanamide resistance gene of SEQ ID NO. 12.

Please delete paragraph [0311] on page 85, and replace it with the following rewritten paragraph:

[0311] 5' ATGTGTCAGAATGAAGTTGAAGTTAATGGATGGACTTCTATG
CCAGCTAATGCTGGAGCTATCTTTGGAGATAAGCCATTTATTAATGAACCAAAG-3' (SEQ ID NO: 53)

Please delete paragraph [0312] on page 85, and replace it with the following rewritten paragraph:

[0312] 5' CAAGAGTCTTAGCAACAACCTGGATCATCAAATGGAACTTAATTTCTT
CAATAGAAAGAGCCTTTGGTTCATTAATAAATGGCTTATCTC-3' (SEQ ID NO: 54)

Please delete paragraph [0313] on page 85, and replace it with the following rewritten paragraph:

[0313] 5' GATCCAGTTGTTGCTAAGACTCTTGATTATGCTAAGGCTGTTCTTCAT
CCAGAACTTTTAATCATTCTATGAGAGTTTATCATTATGGAATG-3' (SEQ ID NO: 55)

Please delete paragraph [0314] on page 86, and replace it with the following rewritten paragraph:

[0314] 5' GGGCCCAAGTAATTGGAGAAAGAGCAGCAGCTTGTTCTGGAAATTGTTG
CTTAGTAATAGCCATTCCATAATGATAAACTCTCATAG-3' (SEQ ID NO: 56).

Please delete paragraph [0315] on page 86, and replace it with the following rewritten paragraph:

[0315] This first gene part was re-amplified with the primers 5'-GGA TCC ATG TGT CAG AAT GAA GTT GAA G-3' (SEQ ID NO: 57) and 5'-GGG CCC AAG TAA TTG GAG AAA GAG C-3' (SEQ ID NO: 58).

Please delete paragraph [0317] on page 86, and replace it with the following rewritten paragraph:

[0317]
5' GGGCCCTTACTTGTCTTCTTCATGATCTTGGAAGCTGAAGAGAATCTTACTGCTACTAGAATGTCTTTGA
TATTTATGGAGGAATTAAGGCTC-3' (SEQ ID NO: 59)

Please delete paragraph [0318] on page 86, and replace it with the following rewritten paragraph:

[0318]
5' CATGTCTAATAATAGCTTCAGCAGCAGCTTCAGCTTGATCAACAGTAGCTCCGAAATCCTTAAGAACAGAAAG
AGCCTTAATTCCTCCATAAATATC-3' (SEQ ID NO: 60)

Please delete paragraph [0319] on page 86, and replace it with the following rewritten paragraph:

[0319]
5' GCTGCTGAAGCTATTATTAGACATGAAGATATGGGAGTTGATGGAAGTATTACTTATATTGGACAACCTATTC
AACTTGCTACTACTTATGATAATAC-3' (SEQ ID NO: 61)

Please delete paragraph [0320] on page 86, and replace it with the following rewritten paragraph:

[0320]
5' GCAGTATTAATTTGAGCCCTAGTTTCATCATGAACAAGTTTACCAAATCCTTAACATGTGGATGAAATCCAG
TATTATCATAAGTAGTAGCAAGTTG-3' (SEQ ID NO: 62)

Please delete paragraph [0321] on page 86, and replace it with the following rewritten paragraph:

[0321]
5' GAAACTAGGGCTCAAATTAATACTGCTTATCCAAGACTTAAGTGGTGTACATTCTTTCTGGAGTTATTAGAA
AGGAAGAACTATTAAGCCATGG-3' (SEQ ID NO: 63)

Please delete paragraph [0322] on page 86, and replace it with the following rewritten paragraph:

[0322] 5' GAGCTCTTATTGTCCATCTGGAGTTCAGCTTCAATTCCTTATCAAAATC
AACAAGATGAGTAGAATGACACCATGGCTTAATAGTTTCTTCCTTTC-3' (SEQ ID NO: 64).

Please delete paragraph [0323] on page 86, and replace it with the following rewritten paragraph:

[0323] The PCR product was re-amplified with the primers 5'-GGG CCC TTA CTT
GTC TTC TTC ATG-3' (SEQ ID NO: 65) and 5'-GAG CTC TTA TTG TCC ATC TGG
AGT-3' (SEQ ID NO: 66). The sequence of the ligated DNA fragments representing the
codon-optimized gene is shown in SEQ ID NO. 15.

Please delete paragraph [0334], beginning on page 91 bridging to page 92, and replace it with the following rewritten paragraph:

[0334] Transformed shoots, generated by infecting potato leaf explants as described above, could be grouped into two different classes. The first class of shoots (55 of 193) was phenotypically indistinguishable from control shoots transformed with LBA::pBI121. The second class of shoots (138 of 193) displayed an IPT phenotype. Shoots of the latter class were stunted in growth, contained only very small leaves, displayed a light-green to yellow color, and were unable to root upon transfer to hormone-free media. To confirm that shoots with an IPT phenotype contained the IPT gene stably integrated in their genomes, all shoots were transferred to Magenta boxes containing MS medium supplemented with 3% sucrose and timentine 150 mg/L, allowed to grow for 3 to 4 additional weeks, and used to isolate DNA. This plant DNA served as template in PCR reactions with an oligonucleotide pair designed to anneal to the IPT gene: 5'- GTC CAA CTT GCA CAG GAA AGA C-3' (**SEQ ID NO: 67**), and 5'- CAT GGA TGA AAT ACT CCT GAG C-3' (**SEQ ID NO: 68**). This PCR experiment confirmed a strict correlation between IPT phenotype and presence of the *IPT* gene. A second PCR experiment was carried out to test whether IPT-free plants did not contain any other backbone sequences. Because the IPT expression cassette is positioned close to the left border-like sequences, the oligonucleotide pair for this experiment was designed to anneal to backbone sequences close to the right border-like sequence: 5'- CAC GCT AAG TGC CGG CCG TCC GAG-3' (**SEQ ID NO: 69**), and 5'-TCC TAA TCG ACG GCG CAC CGG CTG-3' (**SEQ ID NO: 70**). Data from this experiment confirm that plants that are positive for the *IPT* gene are also positive for this other part of the backbone.

Please delete paragraph [0335] on page 92, and replace it with the following rewritten paragraph:

[0335] Instead of the frequently used bacterial terminator of the nopaline synthase gene, a new sequence derived from a food source was used to terminate transcription of a selectable marker gene. This terminator is the yeast alcohol dehydrogenase-1 (ADH1) terminator (Genbank accession number V01292, SEQ ID NO. 4). Surprisingly, this specific yeast terminator was shown to function effectively in plant cells by Agro-infecting potato stem explants with different binary vectors that carry an intron-containing *GUS* gene operably linked to the Ubi7 promoter and followed by either that terminator or the yeast CYC1 terminator. Five days after infection, high levels of transient GUS expression were monitored with the ADH1 terminator, whereas almost no GUS expression was detected with the CYC1 terminator. To terminate transcription of a desired polynucleotide in dicotyledonous plant species, the potato Ubiquitin-3 terminator was used (SEQ ID NO.:5). For transcriptional termination in monocotyledonous plant species, a new terminator was amplified from DNA of the rice variety "Lemont", where it is associated with the actin-1 gene, with the primer set: 5'- GGATCCTCGTCATTTACTTTTATCTTAATGAGC -3' (SEQ ID NO: 71) and 5'- GAATTCACATTATAAGCTTTATATTACCAAGG -3' (SEQ ID NO.:6 72). Functional activity of this rice terminator was demonstrated by operably linking it to a promoter-GUS fusion. Five days after infecting bentgrass seedlings with an *Agrobacterium* strain containing the resulting expression cassette between borders of a binary vector, transient GUS expression levels were equally high as with a control experiment based on a similar vector carrying the frequently used terminator of the bacterial nopaline synthase gene.

Please delete paragraph [0337], beginning on page 93 bridging to page 94, and replace it with the following rewritten paragraph:

[0337] For monocotyledonous plants, a promoter was developed that resembles the sugarcane ubiquitin-4 promoter. The sequence of this small promoter, designated UbiN, is shown in SEQ ID NO.:9; its homology with the corresponding part of the original Ubiquitin-4 promoter is shown in Figure 3. The functional activity of UbiN was assessed by first inserting it between a small HindIII – SalI 0.2-kbp DNA fragment (SEQ ID NO.: 10) isolated from a modified maize matrix attachment region using the primer set: 5'- AAG CTT AAT AGC TTC ACC TAT ATA ATA -3' (SEQ ID NO: 73), and 5'- GTC GAC GGC GTT TAA CAG GCT -3' (SEQ ID NO: 74), and a modified EcoRI – BamHI 1.4-kbp fragment containing an intron associated with a sugarcane ubiquitin gene, using the primer set 5'- GAA TTC CCT TCG TCG GAG AAA TTC ATC GAA G -3' (SEQ ID NO: 75), and 5'- GGA TCC CTG CAA GCA TTG AGG ACC AG -3' (SEQ ID NO.: ~~44~~ 76). The fused DNA fragments were then operably linked to the *CAH* gene followed by a terminator, and a binary vector containing this expression cassette was used to Agro-infect bentgrass seedlings as described in Example 1. Vigorously growing calli demonstrated that the sugarcane-derived promoter is effective in promoting transgene expression.